Environmental Health Perspectives
Vol. 31, pp. 151-159, 1979

Microtus oeconomus (Rodentia), a Useful Mammal for Studying the Induction of Sex-Chromosome Nondisjunction and Diploid Gametes in Male Germ Cells

by A. D. Tates*

Preliminary data indicate that chemicals can also increase the frequency of sex-chromosome nondisjunction. Positive results — which certainly need further confirmation — have been obtained for MMS, *p*-fluorophenylalanine, vincristine, procarbazine, carbendazim, and bleomycin. Nocodazole, benomyl, colcemid, 6-mercaptopurine, and halothane were all negative at the concentrations tested. For the induction of diploid spermatids positive results were only obtained for MMS and parafluorophenylalanine.

In view of the results obtained, the Microtus system is considered a very useful tool for analyzing factors contributing to the high frequency of aneuploidy and triploidy among abortuses and of aneuploidy in liveborn infants of men.

A method is described for the detection of sex-chromosome nondisjunction and diploid spermatids in male germ cells of the field vole *Microtus oeconomus*. The method is based on the unique distribution pattern of heterochromatin in Microtus cells, which makes it possible to identify X and Y chromosomes in early spermatids with a simple C-banding procedure. Slide preparation is easy. Scoring of early spermatids for extra sex-chromosomes is simple and 2000-4000 cells per hour can be examined. With the Microtus system it has now been demonstrated that radiation of spermatocyte stages with doses of 50, 100 and 200 R results in a higher frequency of sex chromosome nondisjunction and of diploid gametes. Both types of aberrant gametes can be produced during the first and second meiotic division.

Introduction

Cytogenetic studies of human abortuses indicate that the frequency of chromosome anomalies among clinically recognizable spontaneous abortions may well be as high as 50% (*1*). Trisomies account for about 50% of all chromosome anomalies among abortuses and triploids constitute the third largest group of chromosomal defects. In newborn infants chromosomal anomalies occur with a frequency of 0.6% and about 60% of these abnormalities are attributable to numerical aberrations of sex chromosomes and autosomes (*2*). In the light of these observations it is considered of great practical interest to explore which factors might result in an increase of this high spontaneous load.

The effects of ionizing radiation and mutagenic chemicals were studied with a new mammalian assay system involving the field vole, *Microtus oeconomus*. *Microtus* is eminently suited for cytological studies on nondisjunction of sex chromosomes in male germ cells because of its unique distribution pattern of heterochromatin (*3*, *4*). With the C-banding technique that stains specifically for heterochromatin, one can demonstrate that the Y chromosome of Microtus is completely heterochromatic. The X-chromosome, which exhibits a block of centromeric heterochromatin that is larger than that of the autosomes, can also be recognized. This special distribution pattern of the heterochromatin in Microtus cells makes it possible to identify the X and

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Y chromosomes in postmeiotic germ cells, particularly in the early spermatid stage. In this paper I describe the assay system, and its use in studying the effect of ionizing radiation and environmental chemicals on sex-chromosome nondisjunction and the production of diploid spermatids.

Materials and Methods

Animals

The breeding colony was started in 1974 with two pairs, one pair had three young. They were captured in the wild by Dr. K. Fredga in Abisko (Lapland), Sweden. The animals are kept in standard Macrolon laboratory cages (42 × 26 × 15 cm) at 20°C and a relative humidity of 65-70%. Cages contain a mixture of peatmoss, sterilized sawdust and some hay. They are cleaned once in a fortnight with hot water and sometimes autoclaved. It is advisable not to use disinfectants. Feed consists predominantly of mixed grains (buckwheat, oats, barley, rye, sunflower seeds) and standard laboratory pellets for guinea pigs. In summer feed is sometimes supplemented with fresh dandelions, whereas in winter apples are given. Water is given *ad libitum*. The animals are predominantly monogamous, and breeding pairs are made when females and males are 3 and 4 months old, respectively. The male is introduced to the female when she is on heat (post-partus-oestrus mating system). Gestation period is 21 days, litter size varies from 2-8 and weaning time is 3 weeks. To avoid excessive aggression, litters are never mixed. The mean weight of adult animals is 69 g ± 11.

Technical Requirements

Apparatus needed is a good quality light microscope, a bench centrifuge (about 1000 rpm), small scissors and forceps for dissection of testis and rupturing of seminiferous tubules, small glass petri dishes, glass pipettes, containers and centrifuge tubes for balanced salt solution, fixative, and staining solution, glass microscope slides and coverslips, and an infrared lamp.

Chemical materials required are sodium citrate solution (2.2%), ethyl alcohol, glacial acetic acid, $Ba(OH)_2 \cdot 8H_2O$ as 1% solution freshly prepared, standard saline citrate (2×) (SSC) solution, De Pe X mounting medium, Giemsa R66 stain, and Leishman stain.

Description of Slide Preparation and Analysis

The procedure for slide preparation is as follows. Ether-anesthesized males are first sacrificed by cervical dislocation. The testes are dissected, the testis tunica removed, and tubuli seminiferi are washed several times in 2.2% (w/v) trisodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) to remove fat. With curved forceps the contents are pushed gently out of the tubuli. The cellular material (with the exception of clearly visible remnants of tubuli) is transferred to a centrifuge tube with 2.2% sodium citrate solution. This suspension is centrifuged for 10 min at 180 *g*, the supernatant removed, and the pellet resuspended in 2.2% sodium citrate. This step is repeated twice. Next 1 ml freshly prepared fixative is added dropwise to the pellet, (3 parts ethyl alcohol to 1 part glacial acetic acid) while flicking the tube vigorously after each drop. More fixative is added, but no longer dripwise. After 10 min, cells are centrifuged for 10 min at 180 *g*. The supernatant is removed and cells resuspended in the fixative; this step is repeated twice, the cells being allowed to remain in fixative for at least half an hour. Two drops of concentrated cell suspension are allowed to fall from a normal Pasteur pipette on an ether-alcohol cleaned slide. When drops have maximally expanded and when Newton rings begin to appear, the investigator must blow gently on the slide to accomplish a flattening of the cells. The blowing is preferentially carried out under a Philips infrared lamp.

Treatment of slides follows this procedure. The 14-day-old slides are allowed to stand for 15 min in a freshly prepared 1% $Ba(OH)_2 \cdot 8H_2O$ solution at room temperature, rinsed three times with distilled water, and rinsed briefly with methanol and again with distilled water. The slides are then treated for 4 hr in a 2 × SSC solution at 65°C and rinsed briefly with distilled water. Staining is carried out for 10 min in a mixture of 4 ml Giemsa R66 and 96 ml Leishman buffer at pH 6.8. After washing with distilled water, the preparations are dried and mounted in De Pe X. Preparations can also be examined directly without mounting. This method gives more reproducible results than that described in an earlier paper (*3*).

Analysis of slides. Evidence for spontaneous and induced nondisjunction gametes and diploid gametes is obtained by analyzing in each animal 1000-2000 spherical nuclei of early spermatids for the presence of extra sex chromosomes. In properly stained slides two types of early spermatids occur in equal proportions (Fig. 1). The first type has a distinct darkly staining compact granule which is interpreted as the Y chromosome in interphase (*3*). The second type contains a somewhat larger, more diffuse and often lighter stained body which sometimes contains one tiny darker stained granule at the periphery. The latter body is interpreted as the X chromosome or a structure associated with the X chromosome. The tiny granule probably represents the centromere of

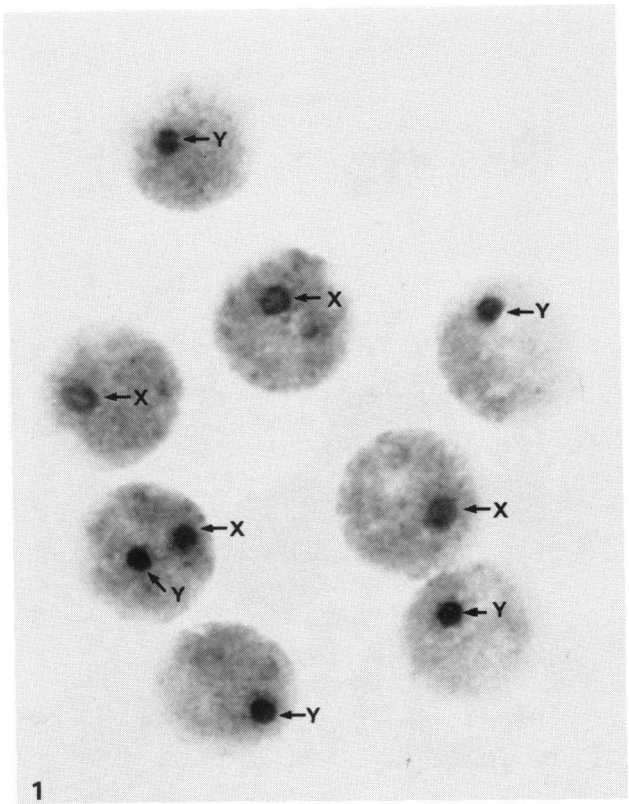

FIGURE 1. Cyst with nuclei of eight early spermatids. Note the presence of cells with X and Y bodies. One spermatid has an X and an Y body. The latter cell is a typical example of a nondisjunction spermatid.

the X chromosome. Nondisjunction spermatids are recognized as early spermatids having two intranuclear bodies (XY, XX or YY). In XY spermatids the nondisjunction event has occured during the first reduction division, whereas cells with two X or two Y bodies are considered to be indicative for second reduction division nondisjunction (Fig. 1; Table 1). According to genetic theory, one can expect equal numbers of ND spermatids with two sex chromosomes and without sex chromosomes. Spermatids without sex chromosomes have indeed been observed but they are not used for statistical treatment of the data because it is impossible to verify whether a cell without a sex chromosome is improperly stained or whether it really is a nondisjunction gamete.

When slides are properly made it can be observed that a sizeable fraction of early spermatids occur in groups or cysts. Cysts consist of daughter cells which differentiate synchronously and are interconnected by intercellular bridges (usually no longer detectable on the slide). Within a cyst all nuclei are of the same size, but between cysts the size of the nuclei differs considerably. This is due to the fact that the size of the nucleus of an early spermatid decreases during its differentiation into a late spermatid. Autoradiographic studies in this laboratory have indicated that the differentiation period from early to late spermatid lasts four days.

Within a cyst one can sometimes observe a nucleus with two sex chromosomes which is markedly larger than that of the other nuclei in the cyst. Cytospectrophotometric DNA measurement of the large nuclei have indicated that such cells are in fact diploid (P. L. Pearson and M. Vander Ploeg, personal

Table 1. Exceptional spermatids that are recorded in the experiments.

Cell type	Source	
Nondisjunction spermatids ($n + 1$)		
Cells with the XY configuration	Nondisjunction during the first meiotic division	XY, XX and YY cells are always pooled in the experiments
Cells with the XX configuration	Nondisjunction during the second meiotic division	
Cells with the YY configuration		
Diploid spermatids ($2n$)		
Cells with the XY configuration	Unknown event during the first meiotic division	XY, XX and YY cells are always pooled in the experiments
Cells with the XX configuration	Unknown event during the second meiotic division	
Cells with the YY configuration		

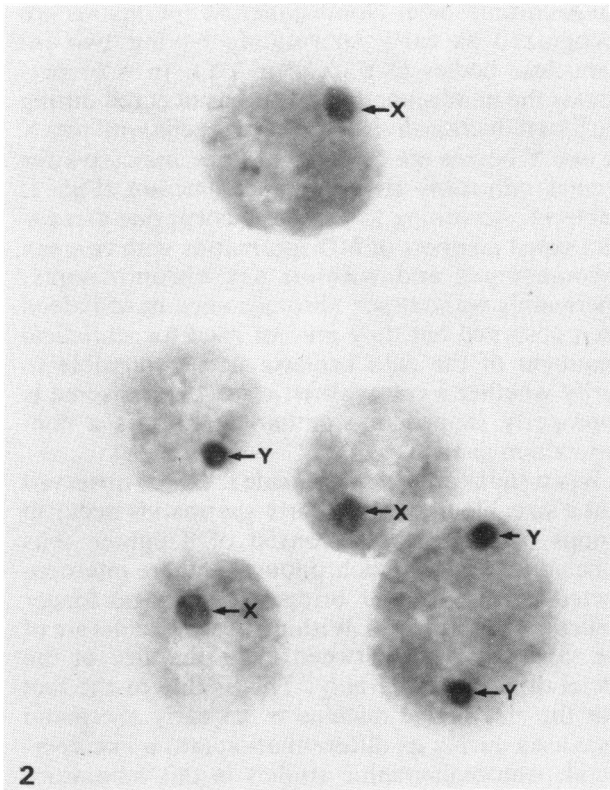

FIGURE 2. Four normal early spermatids plus one diploid spermatid with two Y bodies.

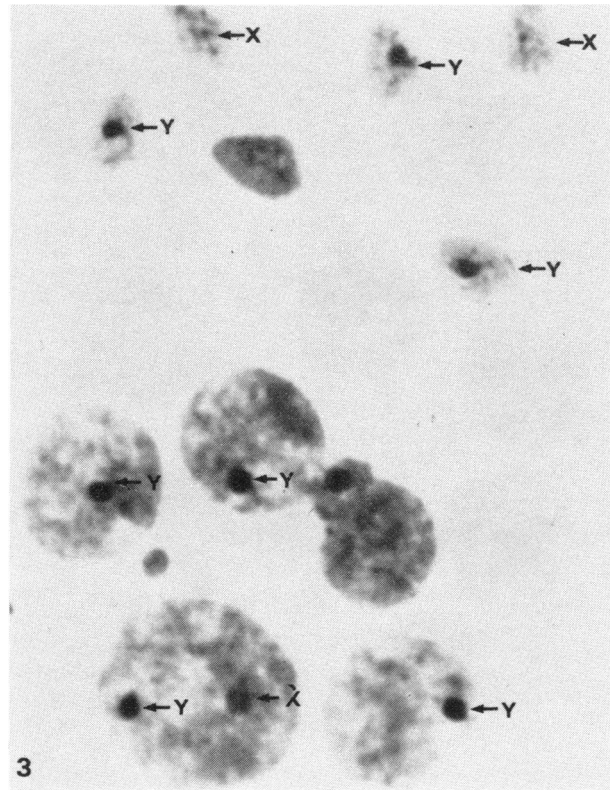

FIGURE 3. A diploid spermatid with the XY configuration can be seen in the lower left corner. The upper part of this figure shows nuclei of six almost mature spermatozoa. Three spermatozoa clearly show the presence of the Y body.

communication). As is the case for nondisjunction spermatids, the diploid spermatids also occur in three types: XY, XX or YY. These three types are also listed in Table 1, and examples of such cells are shown in Figures 2-4. In practice, the mixing up of nondisjunction spermatids and diploid spermatids can be avoided by restricting the scoring to spermatids occurring in cysts with four or more cells.

Statistical Treatment. For the statistical analysis of the data presented in this paper each treated animal is considered as one experimental unit for which the frequency of nondisjunction spermatids and diploid spermatids can be determined. Experimental units are put together in experimental groups. Normally, an experimental group corresponds to the group of animals that was analyzed at a particular sampling time after treatment. For the detection of significant differences between experimental and control groups, p values (one-sided) were computed from the normal approximation of the Wilcoxon two sample statistic (with correction for both ties and continuity). The p values for the experiments with chemicals are given in Table 3.

Chemicals Tested

The following chemicals were tested: methyl methanesulfonate (MMS; Schuchardt), procarbazine (Natulan, Hoffmann-La Roche), vincristine (Oncovin, Lilly), bleomycin (lundbeck), 4-fluoro-dl-β-phenylalanine (PFPA, BDH), carbendazim (MBC, gift Dr. J. P. Seiler, Seitzerland), halothane (Fluothane; ICI), Colcemid (Ciba), Nacodazol (gift from Janssen Pharmaceutica, Belgium), benomyl (Benlate; duPont), 6-mercaptopurine (Merck).

With the exception of carbendazim and halothane, application of test compounds was by intraperitoneal injection. Carbendazim was dissolved in 2% gumarabic solution and administered peroral by gavage. Halothane was administered in gas form (mixed with N_2O and air from 9 a.m. till 4:30 p.m. during 10 successive days.

Results and Discussion

Effect of Ionizing Radiation

The full results and discussion of a study on the effects of ionizing radiation on sex-chromosome

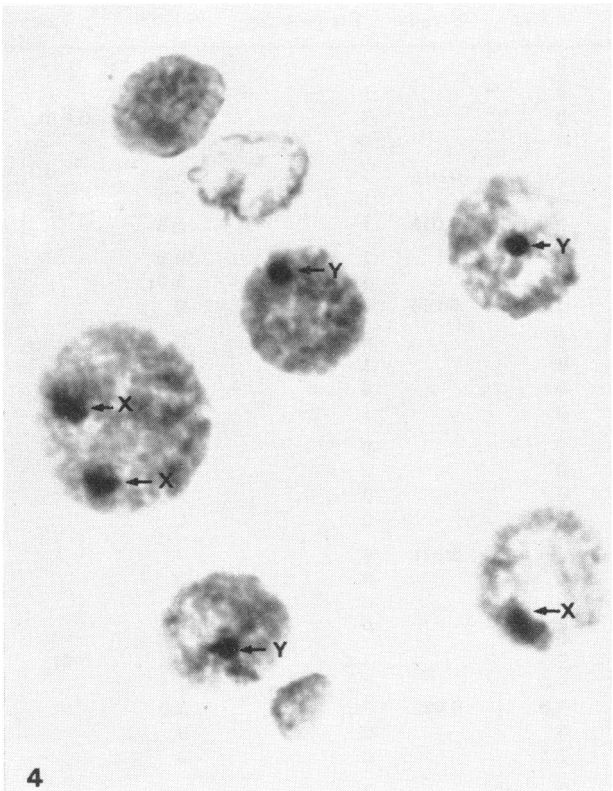

FIGURE 4. Example of a diploid spermatid with the XX configuration.

nondisjunction and the production of diploid gametes form the subject of a separate publication (5). Here only data for five sampling times after irradiation with 50, 100 and 200 R are presented (Figs. 5 and 6). The cell stages sampled at these times are in Figure 7. The data in Figure 5 clearly indicate that at all dose levels there is a significant increase of the frequency of nondisjunctional spermatids. The exceptional spermatids are of the XY, XX and YY type, indicating that nondisjunction for the sex chromosomes is induced during both reduction divisions. At present, no firm conclusions can be drawn with respect to the dose-effect relationships at the various sampling times and the existence of cell stage specific differences in sensitivity for nondisjunction induction.

Table 2. Control frequencies of nondisjunction spermatids and diploid spermatids (cumulative control from radiation experiments).

	Nondisjunction spermatids		Diploid spermatids	
Spermatids sampled /animal	Frequencies	Mean frequency /10,000	Frequencies	Mean frequency /10,000
2000	0,0,0,0,0,0,0,0,0,0	0.5	0,0,0,0,1,1,1,1,1,1,	2.6
3000	0,0,0,0,0,1		0,0,0,1,1,3	
4000	2		0	

Table 3. Effect of chemical mutagens on the induction of sex-chromosome nondisjunction and diploid spermatids.

				Nondisjunction spermatids			Diploid spermatids		
Compound	Concn, mg/kg	Sampling time, days	Spermatids sampled/ animal	Frequencies	Mean frequency /10,000	*p* value	Frequencies	Mean frequency /10,000	*p* value
Compounds with a positive effect in one or more prespermatid stages									
MMS	50	2	2000	0,0,0,0,0	0		0,0,1,1,4	6.0	
		5	2000	0,0,0,0,1	3.0		0,0,0,1,2	3.0	
		8	2000	0,0,0	0		1,2,2	8.3	0.013
		11	2000	1,2	7.5	0.002	0,4	10.0	
		14	2000	0,1	2.5		2,5	17.5	0.016
Procarbazine (Natulan)	100	4	2000	2	10.0	0.011	5	25.0	0.050
		8	2000	0	0		0	0	
		11	2000	0,1	2.5		0,0	0	
		12	2000	0	0		2	10.0	
		13	2000	0	0		2	10.0	
		14	2000	0	0		0	0	
		15	2000	0	0		3	15.0	0.050
	200	4	2000	0	0		1	5.0	
		8	2000	0	0		2	10.0	
		11	2000	0,0	0		1,4	12.5	0.036
		12	2000	0	0	2		10.0	
		14	2000	3	15.0	0.011	0	0	
		15	2000	0	0		1	5.0	
	400	10	2000	0,0,1	1.7		0,0,2	3.3	

continued

Table 3 (Cont'd)

Compound	Concn, mg/kg	Sampling time, days	Spermatids sampled/ animal	Nondisjunction spermatids: Frequencies	Nondisjunction spermatids: Mean frequency /10,000	Nondisjunction spermatids: p value	Diploid spermatids: Frequencies	Diploid spermatids: Mean frequency /10,000	Diploid spermatids: p value
Vincristine (Oncovin)	0.05	1	2000	0	0		1	5.0	
		2	2000	0	0		1	5.0	
		8	2000	0	0		3	15.0	0.050
		17	2000	0	0		0	0	
	0.10	7	2000	1	5.0	0.016	1	5.0	
		8	2000	0	0		1	5.0	
		9	2000	1	5.0	0.016	1	5.0	
	0.20	12	2000	0	0		2	10.0	
		13	2000	0	0		1	5.0	
		14	2000	1	5.0	0.016	0	0	
Bleomycin	0.05	4	1000	0	0		0	0	
		7	1000	0	0		1	10.0	
		11	1000	0	0		4	40.0	0.050
		14	1000	0	0		1	10.0	
	0.10	4	1000	0	0		0	0	
		7	1000	0	0		0	0	
		11	1000	0	0		0	0	
		14	1000	0	0		0	0	
	0.50	4	1000	1	10.0	0.011	0	0	
		7	1000	0	0		0	0	
		11	1000	0	0		0	0	
		14	1000	0	0		0	0	
4-Fluoro-*dl*-β-phenylalanine	2 × 150 (24 hr)	8	2000	0,1	2.5		3,4	17.5	0.011
		9	2000	0,2	5.0		0,1	2.5	
		10	2000	1,1	5.0	0.002	0,2	5.0	
		11	2000	0	0		0	0	
		12	2000	0,1	2.5		0	0	
Carbendazim (MBC)	2 × 250 (24 hr)	1	2000	0	0		2	10.0	
		4	2000	0,0	0		0,0	0	
		6	2000	0,1	2.5		0,1	2.5	
		8	2000	0,0	0		1,1	5.0	
		10	2000	1	5.0	0.016	1	5.0	
		12	2000	0,0	0		0,2	5.0	
		14	2000	0,0	0		0,1	2.5	
		16	2000	0,0	0		0,1	2.5	
Halothane	0.02%	11	2000	0,0	0		0,2	5.0	
		14	2000	0	0		4	20.0	0.051
	2% N_2O; rest air	15	2000	0	0		1	5.0	
		18	2000	0,0	0		0,0	0	
		21	2000	0	0		1	5.0	
		23	2000	0	0		3	15.0	0.050
Compounds without positive effects									
Colcemid	3.7	3	2000	0,0,0,0,1	1.0		0,0,1,4,5	10.0	
		6	2000	0,0,0,0,0,0	0		0,0,0,1,1,4	5.0	
Nocodazol	120	6	1000	0,0,0	0		0,0,1	3.3	
		9	1000	0,0,0	0		0,0,1	3.3	
		12	1000	0,0,0	0		0,0,0	0	
Benomyl (Benlate)	50	10	2000	0,0	0		0,1	2.5	
	250	11	2000	0,0	0		0,0	0	
		12	2000	0,0	0		0,0	0	
		13	2000	0,0	0		0,1	2.5	
		14	2000	0,0	0		0,1	2.5	
	500	10	2000	0	0		0	0	
6-Mercaptopurine	75	8	2000	0,0	0		0,3	7.5	
		10	2000	0,0	0		0,0	0	
		13	2000	0,0	0		0,0	0	
		14	2000	0,0	0		0,0	0	
		15	2000	0,0	0		0,0	0	
		16	2000	0,0	0		0,0	0	

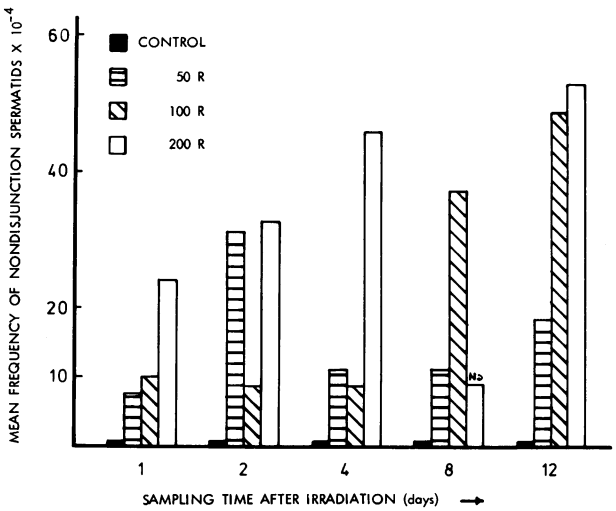

FIGURE 5. Mean frequencies of nondisjunction spermatids at five different sampling times after irradiation of prespermatid stages with x-ray doses of 50, 100, and 200 R. (n.s. = not significant).

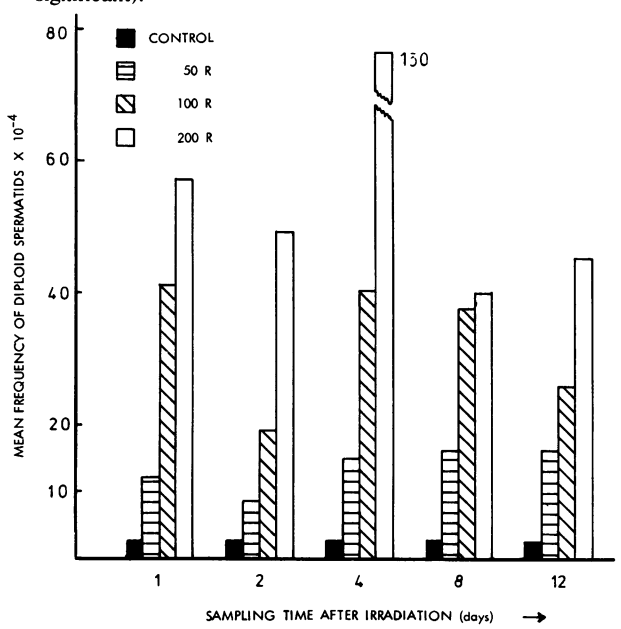

FIGURE 6. Mean frequencies of diploid spermatids at five different sampling times after irradiation of pre-spermatid stages with x-ray doses of 50, 100, and 200 R.

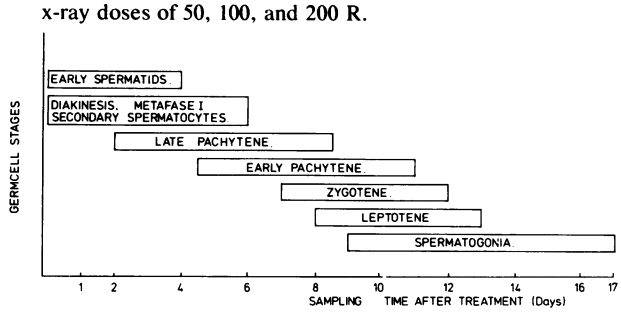

FIGURE 7. Germ cell stages that are sampled at a particular time interval after irradiation.

Without exception, the three doses of radiation significantly increased the frequency of diploid spermatids at all sampling times (Fig. 6). Diploid spermatids were of the XY, XX and YY type. At all sampling times the induction of diploid spermatids is clearly dose-dependent, and the shape of the dose-effect relationship at different sampling times is more uniform than that observed for the induction of non-disjunctional spermatids.

Effects of Chemicals

I want to emphasize strongly the preliminary nature of the results obtained with chemicals. The Microtus colony is not yet large enough to provide large numbers of animals required. Therefore, the effect of chemicals could only be studied with rather small numbers of animals and only one or a few concentrations could be tested. Duplicate experiments have not yet been carried out. As a further economy the cumulative control of the radiation experiments has been used as a control for all experiments with chemicals (Table 2).

Methyl Methanesulfonate. MMS significantly increased the frequency of nondisjunction spermatids in cell stages corresponding to early spermatocyte stages (Table 3). At present, a more precise delineation of the cell stage is impossible. On days 8 and 14 after MMS treatment there was also a significant increase in the frequency of diploid spermatids. It is of interest to mention that chromosome analysis of metaphase II spermatocytes in the mouse provided evidence for nondisjunction induction in early spermatocytes by the same MMS concentration as was used in the Microtus experiment (*6*).

Procarbazine. Procarbazine is an antineoplastic drug used for the treatment of Hodgkin's disease. It is one of the compounds that has extensively been studied in the context of the Environmental Research Program of the Commission of the European Communities. This compound was found to be a potent indirect mutagen and chromosome breaker in a large variety of test systems and it was considered to be of interest to test its possible interference with the normal disjunction of chromosomes. An injection of 100 mg/kg was found to increase significantly the frequency of nondisjunction spermatids on day 4. At the higher concentration of 200 mg/kg a significant increase was found on day 15. The effect of procarbazine on the production of diploid spermatids is very small.

4-Fluoro-dl-β-phenylalanine (PFPA). The amino acid analog PFPA was studied in the Microtus system because it has been shown to induce nondisjunction in microorganisms. PFPA was positive in the D_9J_2 strain of *Saccharomyces cerevisiae* which is

suitable for the detection of disomic spores ($n+1$) produced by meiotic nondisjunction for chromosome VII (*7*). In the *Neurospora crassa* test system, which also monitors meiotic nondisjunction, PFPA was also positive (*8*). These recent studies confirm earlier findings that PFPA can induce aneuploidy in a variety of fungal cultures by interfering with the normal distribution of chromosomes in mitosis (*9-12*).

The present pilot study with the Microtus systems shows that PFPA is also capable of inducing nondisjunction in a mammal. PFPA seems to act in early spermatocyte stages sampled on day 10. That PFPA also seems to interfere with the segregation of a complete set of chromosomes is indicated by the significant increase in the frequency of diploid spermatids on day 8 after treatment.

Benzimidazole Derivatives. Among the three benzimidazole derivatives (benomyl, MBC, Nocodazol) tested in Microtus, only MBC was effective in inducing nondisjunction in young primary spermatocytes (day 10 after treatment). No evidence has been obtained for the induction of diploid spermatids. Benomyl was found to be a strong inducer of mitotic nondisjunction in *Aspergillus nidulans* (*13, 14*). The effect of benomyl on the normal segregation of chromosomes in Aspergillus is most probably due to its interference with spindle formation, because benomyl is readily hydrolyzed to MBC which occupies a key position in the toxicology of benzimidazole pesticides and which is known to be a spindle poison. The spindle poison action of MBC seems to result from an absorption of the carbamate groups to microtubular proteins. This disturbs the formation of the microfibrillar spindle apparatus and nondisjunction and other mitotic disturbances follow. For Nocodazol it is also well documented that it interferes with the structure and formation of microtubules, both in interphase and mitotic cells (*15*).

Halothane. Halothane was tested in the Microtus system because several reports have indicated that it can induce aneuploidy. For example, in *Vicia faba* root tip cells, exposure to clinically useful concentrations of halothane leads to an increase of the spontaneous incidence of aneuploidy and polyploidy (*16*). Several epidemiological studies on operating room personnel have indicated that occurrence of relatively high frequencies of abortions and of congenital abnormalities in their progeny. These effects have mainly been attributed to adverse effects of waste anesthetic gases, in particular halothane (*17-19*). The Microtus males were exposed to halothane during the daytime for a period of 10 consecutive days. The animals were not anesthetized by the concentration of gas used in these treatments. Treated animals were sacrificed from day 11 to 23 after the beginning of the treatment with halothane. When the animals were sacrificed and opened for the dissection of the testes the smell of halothane was clearly noticeable. The experiments which have thus far been carried out have not provided evidence for an effect of halothane on nondisjunction induction. With respect to the induction of diploid spermatids the effect of halothane is on the borderline of significance.

Vincristine. The cytostatic drug vincristine is known to interfere with the spindle apparatus. This is indicated by the finding of "tetraploid" polychromatic erythrocytes following the treatment of bone-marrow of mice with vincristine (*20*). In Microtus, vincristine (0.10 and 0.20 mg/kg) significantly increased the frequency of nondisjunction spermatids in some cell stages but not in others. Obviously, more extensive studies are required before definite conclusions can be drawn with respect to cell stage specificity. There is no clearcut indication for an induction of diploid gametes.

Bleomycin. The antitumor antibiotic bleomycin induces chromosomal aberrations in human leukocytes *in vitro* and in Chinese hamster ovary cells *in vitro* (*21, 22*). It also induced premature chromosome condensation in human leukocytes *in vitro* (which ultimately can be the result of nondisjunction or chromosomal aberrations (*23*). The observation that bleomycin increases the frequencies of univalents in dividing primary spermatocytes of mice that were treated in the spermatogonial stages is a further indication that this compound may interfere with chromosome separation processes (P. P. W. van Buul, personal communication).

I have tested bleomycin in Microtus. It was impossible to use the concentration that induced univalents in the mouse (40 mg/kg) because this concentration is lethal in Microtus. In the latter species, it was only possible to test concentrations below 1 mg/kg. The present pilot study with Microtus provides an indication that bleomycin (0.5 mg/kg) may induce nondisjunction in late spermatocyte stages.

Colcemid. It has long been known that colcemid is capable of inducing nondisjunction in mammalian somatic cells *in vitro*. This will lead to the formation of cells which are polyploid or mono- and trisomic for one or more individual chromosomes (*24-27*). There are no *a priori* reasons to assume that colcemid would not be able to interfere with the disjunction of chromosomes during both meiotic divisions in Microtus males. So far, no positive results have been detected, but this may be due to the fact that only one concentration and just a few spermatocyte stages have been investigated.

6-Mercaptopurine. The antileukemic purine analog 6-mercaptopurine inhibits purine nucleotid

synthesis (*28*). This nonalkylating chemical (dose 150 mg/kg) has been shown to be capable of inducing chromosome breakage in late-differentiating spermatogonia and very early spermatocytes of mice (*29, 30*). There is also some evidence that it may induce nondisjunction in the above germ cell stages, because among 615 F_1 sons of treated males, there were three sterile animals which showed the XYY condition (*31*). This finding prompted me to study the effect of 6-mercaptopurine in Microtus, but until now, no evidence for nondisjunction could be obtained.

I am grateful to Prof. F. H. Sobels for his interest in the project, and to Drs. J. G. P. Tyssen and A. C. Verwey for help with the statistical analysis. The competent technical assistance of Mr. N. De Vogel is gratefully acknowledged. This work was supported by the J. A. Cohen Interuniversity Institute for Radiopathology and Radiation Protection and by Contract No. 139-77-1 ENV N of the European Community Environmental Research Programme.

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